Supplementary Information:

Directed evolution of mesophilic HNA polymerases providing insight into DNA polymerase mechanisms.

Authors and affiliations:

Paola Handal-Marquez[1,2], Leticia L. Torres[1] and Vitor B. Pinheiro[2*]

[1] University College London, Department of Structural and Molecular Biology, Gower Street, WC1E 6BT, London, UK

[2] KU Leuven, Rega Institute for Medical Research, Department of Pharmaceutical and Pharmacological Sciences, Herestraat, 49 – box 1041, 3000 Leuven, Belgium

[*] Corresponding author: e-mail: v.pinheiro@kuleuven.be

Table of Contents:

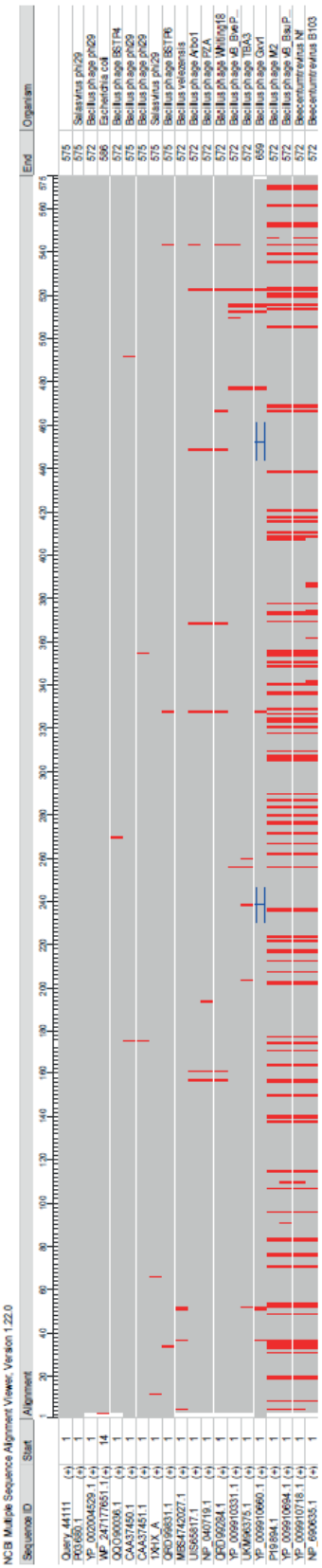

Figure S1. Phi29 DNAP homologues in public databases. Sequences with >80% sequence identity and >80% query cover from a blast search of the Phi29 DNAP protein sequence were selected, aligned using the NCBI Multiple Alignment Tool (Altschul et al., 1990) and viewed using the NCBI'S Sequence Viewer (Rangwala et al., 2021). Mismatches relative to the query (Phi29 DNAP sequence) are shown in red and insertions are indicated by a blue bracket. Only 20 sequences show significant similarity to phi29 DNAP.

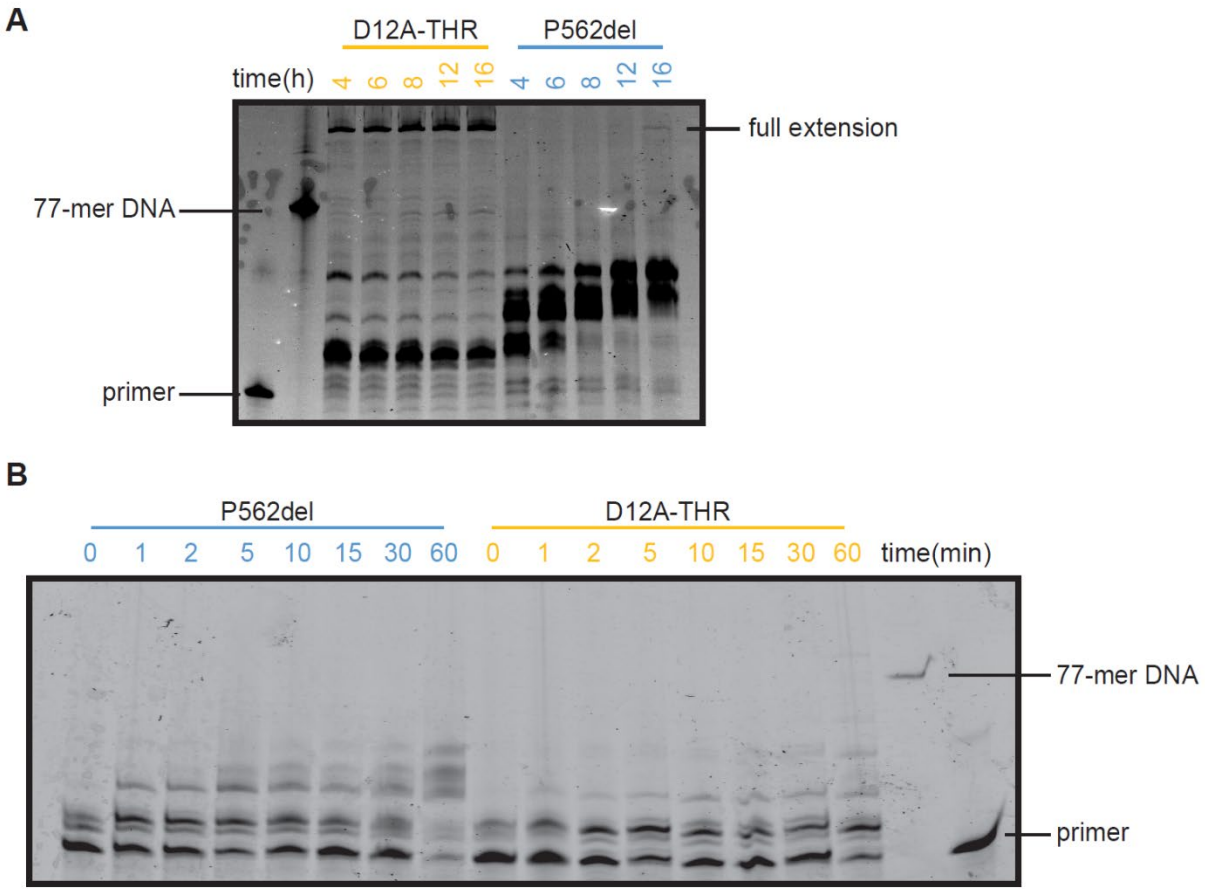

Figure S2. HNA synthesis time courses by D12A-THR and p562del with different templates. Products from primer extensions by D12A-THR and P562 mutants on the TempN-exoR (Table S2) template with incubation times from 4 to 16 h (**A**) as well as on the TempN_2.7_ExoR template (Table S2) with incubation times from 0 to 1 h (**B**) were separated by denaturing PAGE. The TempN_2.7_ExoR template is a modified version of TempN-exoR with 4 substitutions and 1 deletion that reduces the probability of secondary structure formation. Fully extended products (57 hNTP incorporations) are shown. HNA migrates slower than DNA in denaturing PAGE (Torres & Pinheiro, 2018).

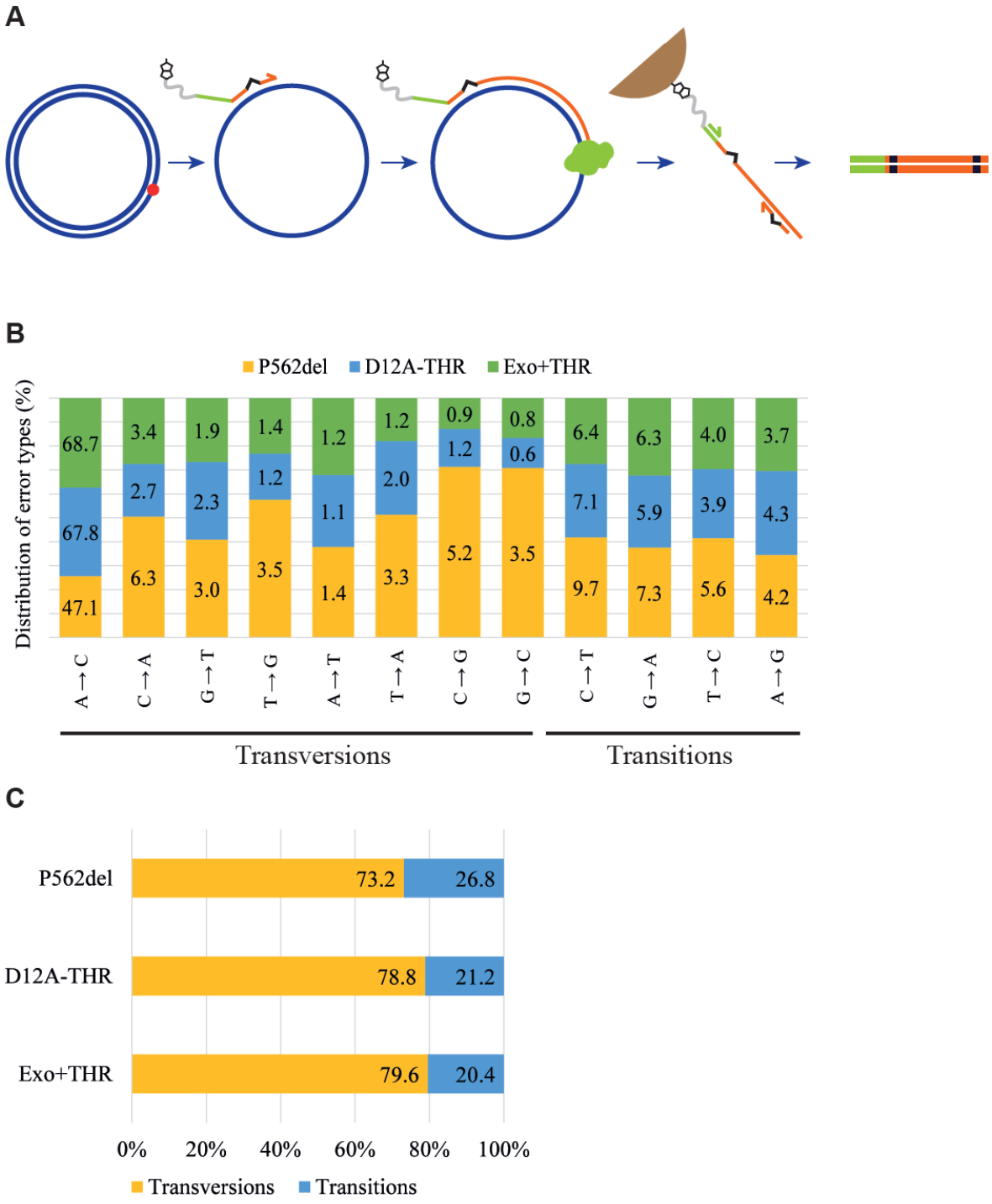

Figure S3. Phi29 DNAP P562del reduced fidelity and increased InDel incorporations rate. (**A**) Workflow of the isothermal polymerase fidelity assay. The red dot marks the

nicking site for single stranded plasmid generation, the non-complementary overhang of the primer for downstream amplification is shown in green and 1 bp mismatches are shown in black. The primer is extended, captured, and purified through biotin-streptavidin pulldown and used as template in a secondary PCR amplification step. (**B**) Distribution and quantification of error (misincorporation) types introduced by each mutant during isothermal DNA replication. Each error type was identified by comparing the isothermal amplification products post-deep sequencing and after their alignment to the Fidelity_ref (Table S2) on a base-to-base manner. The sum of each error type was divided by the total number of misincorporations and multiplied times a 100 to yield the error type percentage displayed. (**C**) The total percentage of inversions and transversions introduced by each mutant.

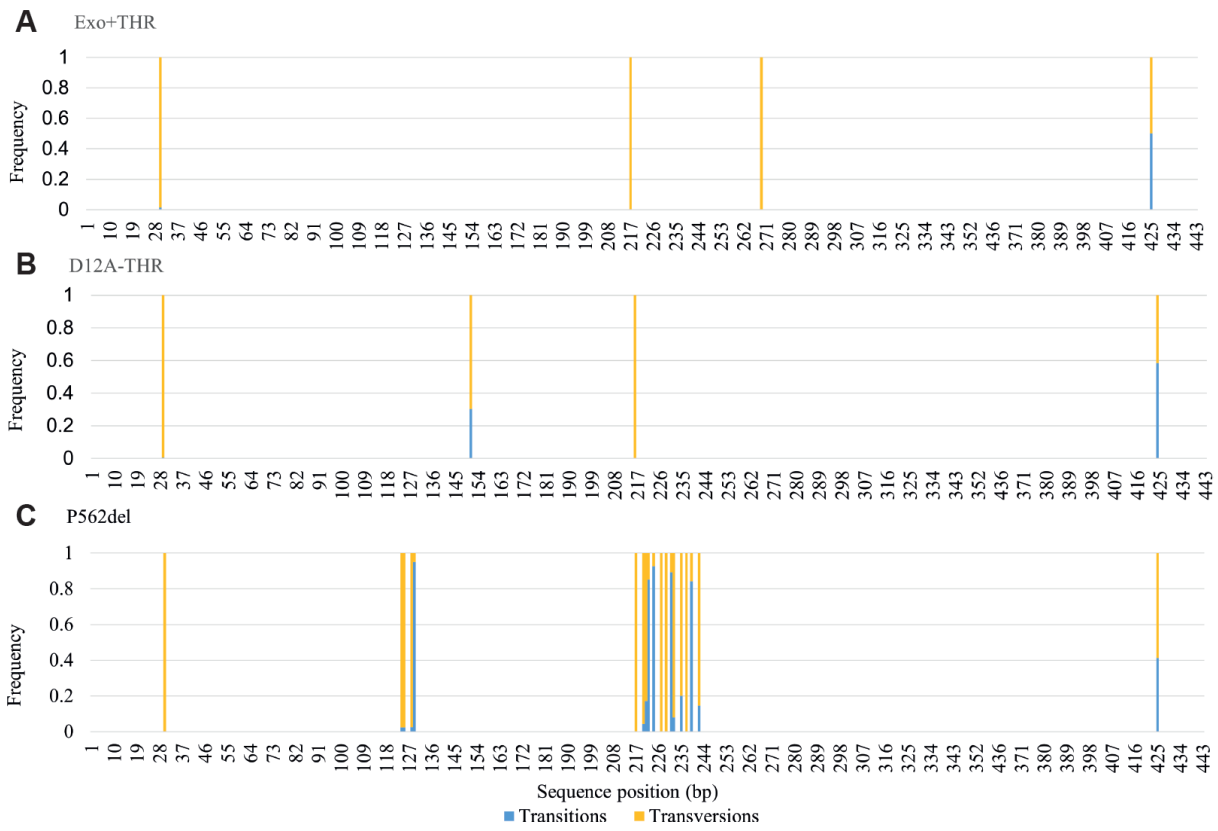

Figure S4. Transition and transversion hotspots introduced by phi29 DNAP variants. The products from the isothermal DNA replication fidelity assays generated by Exo+THR, D12A-THR and p562del were deep sequenced, filtered by quality, trimmed, and aligned. The MSA alignments were used to quantify the abundance of transitions and transversions per position by comparing each of the aligned reads to the Fidelity_ref (table S2) sequence within each the alignment in a base-by-base manner. The total number of transitions and transversions per position was divided by the number of reads to obtain overall frequency scores. Only positions with transitions or transversions with overall frequency scores above 0.5% were selected for visualization. The scores of each error type were divided by the sum of both scores to obtain the frequency value per position and were plotted against the Fidelity_ref length.

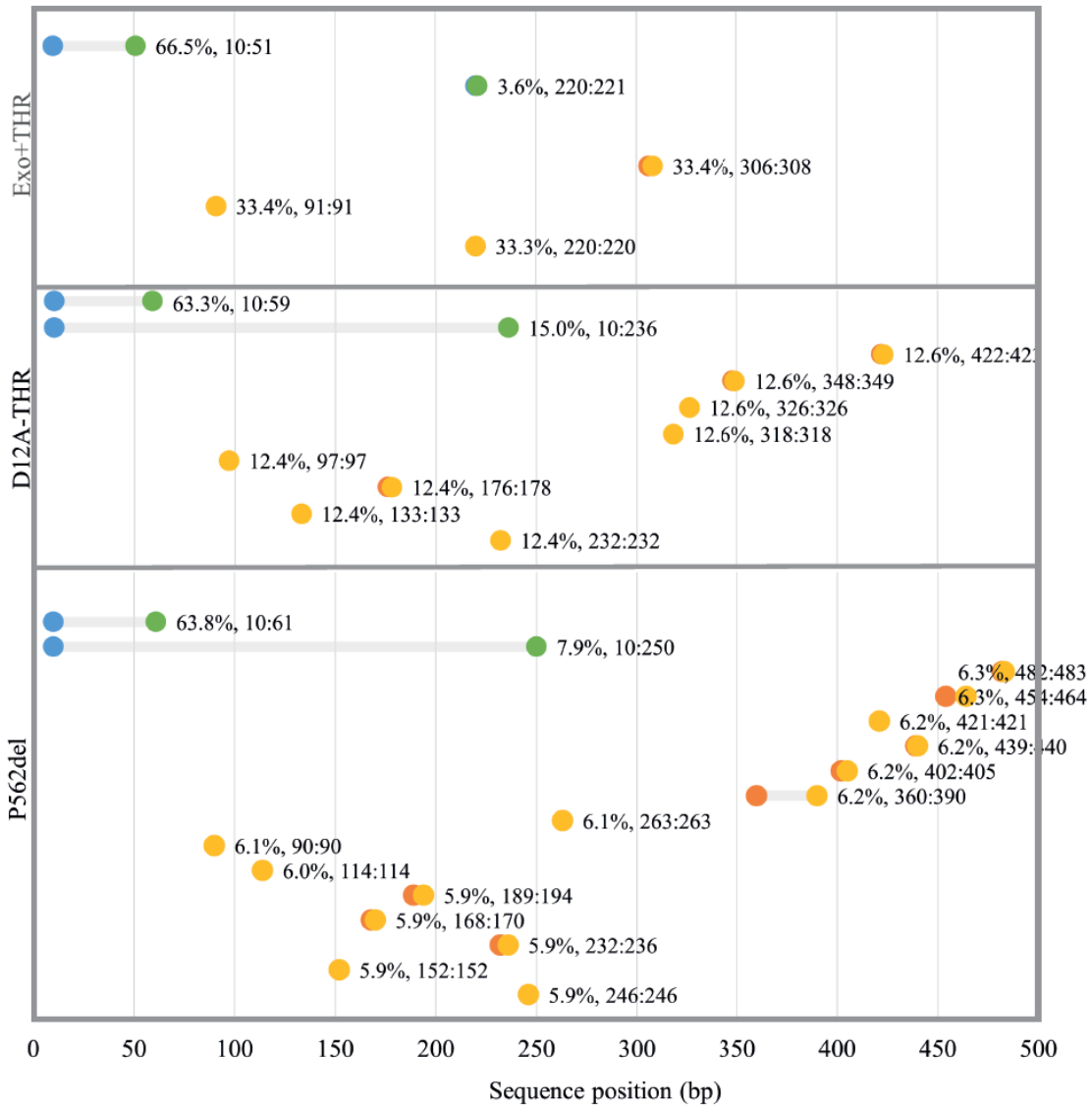

Figure S5. Location of most abundant InDel introductions by each mutant during isothermal DNA replication. Location of deletions (blue to green dots) and insertions (orange to yellow dots) appearing with >5% frequency relative to all the insertions or deletions identified in the MSA of the isothermal amplification products generated by each mutant. The x-axis indicates the sequence length of the template used in the assay/analysis. Blue or orange dots indicate the 'start' of the deletion or insertion respectively, and the green or yellow dots indicate the 'end' of the deletion or insertion

respectively. The percentage values adjacent to the ‘end’ dots represent the abundance of deletions or insertions relative to the total number of deletions or insertions respectively. The percentage values are followed the location of the particular InDel in a range format.

Table S1. Sequences[a] of all the plasmids used in this study.

pET23-P2-D12A-THR
acgcgccctgtagcggcgcattaagcgcggccgctgtggtggttacgcgcagcgtgaccgctacacttgccagcgcc
ctagcgcccgctcctttcgctttcttcccttcctttctcgccacgttcgccggctttccccgtcaagctctaaatcgggggctcc
ctttagggttccgatttagtgctttacggcacctcgaccccaaaaaacttgattagggtgatggttcacgtagtgggccatc
gccctgatagacggtttttcgccctttgacgttggagtccacgttctttaatagtggactcttgttccaaactggaacaacact
caaccctatctcggtctattcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgagctgatttaacaa
aaatttaacgcgaattttaacaaaatattaacgtttacaatttcaggtggcacttttcggggaaatgtgcgcggaacccct
atttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataaccctgataaatgcttcaataatattgaaaaa
ggaagagtatgagtattcaacatttccgtgtcgcccttattccctttttgcggcattttgccttcctgtttttgctcacccagaaa
cgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggta
agatccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaaagttctgctatgtggcgcggtattatcc
cgtattgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgagtactcaccagtca
cagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcg
gccaacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactc
gccttgatcgttgggaaccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgcagcaat
ggcaacaacgttgcgcaaactattaactggcgaactacttactctagcttcccggcaacaattaatagactggatggag
gcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctggagccggtga
gcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacgggg
agtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaactgtca
gaccaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatccttttttgataat
ctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatcttcttga
gatcctttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaag
agctaccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtacttctagtgtagccgtagt
taggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtggctgctgccagtg
gcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggataaggcgcagcggtcgggctgaacgggg
ggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgtgagctatgagaaa
gcgccacgcttcccgaagggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgcac
gagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgt

gatgctcgtcaggggggcggagcctatggaaaaacgccagcaacgcggccttttacggttcctggccttttgctgcgtt
atcccctgattctgtggccttttgcgcgtctgcgttatcccctgattctgatgttctttcctgcgttatcccctgattctgtggataa
ccgtattaccgcctttgagtgagctgcgttatcccctgattctgctgataccgctcgccgcagccgaacgaccgagcgca
gcgagtcagtgagcgaggaagcggaatatcgcctgatgcggtattttctccttacgcatctgtgcggtatttcacaccgc
aatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagtatacactccgctatcgctacgtgactgggt
catggctgcgccccgacacccgccaacacccgctgacgcgccctgacgggcttgtctgctcccggcatccgcttaca
gacaagctgtgaccgtctccgggagctgcatgtgtcagaggttttcaccgtcatcaccgaaacgcgcgaggcagctgc
ggtaaagctcatcagcgtggtcgtgaagcgattcacagatgtctgcctgttcatccgcgtccagctcgttgagtttctccag
aagcgttaatgtctggcttctgataaagcgggccatgttaagggcggttttttcctgtttggtcactgatgcctccgtgtaagg
gggatttctgttcatgggggtaatgataccgatgaaacgagagaggatgctcacgatacgggttactgatgatgaacat
gcccggttactggaacgttgtgagggtaaacaactggcggtatggatgcggcgggaccagagaaaaatcactcagg
gtcaatgccagcgcttcgttaatacagatgtaggtgttccacagggtagccagcagcatcctgcgatgcagatccgga
acataatggtgcagggcgctgacttccgcgtttccagactttacgaaacacggaaaccgaagaccattcatgttgttgct
caggtcgcagacgttttgcagcagcagtcgcttcacgttcgctcgcgtatcggtgattcattctgctaaccagtaaggcaa
ccccgccagcctagccgggtcctcaacgacaggagcacgatcatgcgcacccgtggccaggacccaacgctgccc
gagatctcgatcccgcgaaattaatacgactcactatagggagaccacaacggtttccctctagaaataattttgtttaac
tttaagaaggagatataccatggatcctctagagtcgacctgcaggcatgcaagcttgcggccacacaggagatagtc
atacatgaaacacatgcctcgcaaaaggtatagctgcgcttttgaaaccaccaccaaagttgaagattgtcgtgtttggg
catatggctatatgaacattgaagatcacagcgagtataaaatcggcaatagcctggatgaatttatggcatgggctctg
aaagttcaggccgatctgtattttcacaatctgaaatttgatggtgccttcattattaactggctggaacgcaatggttttaaa
tggtcagcagatggtctgccgaatacctataacaccattattagccgtacgggccagtggtatatgattgatatttgcctgg
gttataaaggcaaacgcaaaattcataccgtgatctatgacagcctgaaaaaactgccgtttccggtgaaaaaaatcg
ccaaagatttcaaactgaccgtgctgaaaggcgatatcgattatcacaaagaacgtccggttggctacaaaattacac
cggaagaatatgcctacatcaaaaacgacattcagattattgcagaagccctgctgattcagtttaaacagggtctggat
cgtatgaccgcaggtagcgatagcctgaaagattttaaagatatcattaccaccaaaaaattcaaaaaagtgttcccga
ccctgagcctgggcctggataaaaaagttcgttacgcatatcgcggtggttttacctggctgaatgatcgctttaaagaaa
aagaaattggcgagggcatggtgtttgatgttaatagcctgtatccggcacagatgtatagccgtctgctgccgtatggtg
aaccgattgtttttgaaggtaaatatgtgtgggatgaggattatccgctgcatattcagcatattcgttgcgaatttgaactga
aagaaggctatattccgaccattcagatcaaacgtagccgcttctataaaggtaacgagtatctgaaaagcagcggtg
gtgaaattgcagatctgtggctgagcaatgttgatctggaactgatgaaagaacactacgatctgtacaacgtggaatat
atcagcggtctgaaattcaaagcaaccaccggtctgttcaaagacttcattgataaatggacctatatcaaaaccacctc

cgaaggtgcaattaaacagctggcaaaactgatgctgaattccctgtatggtaaatttgcaagcaatccggatgtgacc
ggtaaagttccttatctgaaagaaaatggtgcactgggttttcgtctgggtgaagaagaaaccaaagatccggtttatac
cccgatgggtgtgtttattaccgcatgggcacgttataccaccattaccgcagcacaggcatgttatgaccgtattatctatt
gtgataccgatagcattcatctgaccggcaccgaaattccggatgttatcaaagatattgtggatcctaaaaaactgggc
tattgggcacatgaaagcacctttaaacgtgcaaaatatctgcgcccgaaaacctatatccaggatatctatatgaaag
aagtggacggtgaactggttgcaggtagtccggatgattacaccgatatcaaacttagcgttaaatgtgccggtatgac
cgataaaatcaaaaaagaagtgaccttcgagaacttcaaagtgggttttagccgtaaaatgaaaccgaaaccggttc
aggttccgggtggtgttgttctggttgatgataccttttacgatcaaacaccaccaccaccaccactgagatccggctgcta
acaaagcccgaaaggaagctgagttggctgctgccaccgctgagcaataactagcataacccccttggggcctctaaa
cgggtcttgaggggttttttgctgaaaggaggaactatatccggattggcgaatggg

pET23_KOD_DA_Mut

tggcgaatgggacgcgccctgtagcggcgcattaagcgcggccgctgtggtggttacgcgcagcgtgaccgctacac
ttgccagcgccctagcgcccgctcctttcgctttcttcccttcctttctcgccacgttcgccggctttccccgtcaagctctaaa
tcgggggctcccttagggttccgatttagtgctttacggcacctcgaccccaaaaaacttgattagggtgatggttcacgt
agtgggccatcgccctgatagacggtttttcgccctttgacgttggagtccacgttctttaatagtggactcttgttccaaact
ggaacaacactcaaccctatctcggtctattcttttgatttataagggattttgccgatttcggcctattggttaaaaaatgag
ctgatttaacaaaaatttaacgcgaattttaacaaaatattaacgtttacaatttcaggtggcacttttcggggaaatgtgcg
cggaacccctatttgtttatttttctaaatacattcaaatatgtatccgctcatgagacaataaccctgataaatgcttcaata
atattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttattccctttttgcggcattttgccttcctgtttttgct
cacccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtgggttacatcgaactggatct
caacagcggtaagatccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaaagttctgctatgtggc
gcggtattatcccgtattgacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgagta
ctcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtga
taacactgcggccaacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatggggga
tcatgtaactcgccttgatcgttgggaaccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgc
ctgcagcaatggcaacaacgttgcgcaaactattaactggcgaactacttactctagcttcccggcaacaattaataga
ctggatggaggcggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctgg
agccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctaca
cgacggggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattgg
taactgtcagaccaagtttactcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaagatcct

ttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaagg
atcttcttgagatcctttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgcc
ggatcaagagctaccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtacttctagtgt
agccgtagttaggccaccacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtggctg
ctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccggataaggcgcagcggtcgggct
gaacgggggttcgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgtgagct
atgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatccggtaagcggcagggtcggaacagga
gagcgcacgagggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctgacttgagcg
tcgatttttgtgatgctcgtcaggggggcggagcctatggaaaaacgccagcaacgcggccttttacggttcctggcctt
ttgctgcgttatcccctgattctgtggccttttgcgcgtctgcgttatcccctgattctgatgttctttcctgcgttatcccctgattct
gtggataaccgtattaccgcctttgagtgagctgcgttatcccctgattctgctgataccgctcgccgcagccgaacgacc
gagcgcagcgagtcagtgagcgaggaagcggaatatcgcctgatgcggtattttctccttacgcatctgtgcggtatttc
acaccgcaatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagtatacactccgctatcgctacgt
gactgggtcatggctgcgccccgacacccgccaacacccgctgacgcgccctgacgggcttgtctgctcccggcatc
cgcttacagacaagctgtgaccgtctccgggagctgcatgtgtcagaggttttcaccgtcatcaccgaaacgcgcgag
gcagctgcggtaaagctcatcagcgtggtcgtgaagcgattcacagatgtctgcctgttcatccgcgtccagctcgttga
gtttctccagaagcgttaatgtctggcttctgataaagcgggccatgttaagggcggttttttcctgtttggtcactgatgcctc
cgtgtaaggggatttctgttcatgggggtaatgataccgatgaaacgagagaggatgctcacgatacgggttactgat
gatgaacatgcccggttactggaacgttgtgagggtaaacaactggcggtatggatgcggcgggaccagagaaaaa
tcactcagggtcaatgccagcgcttcgttaatacagatgtaggtgttccacagggtagccagcagcatatggtgcaggg
cgctgacttccgcgtttccagactttacgaaacacggaaaccgaagaccattcatgttgttgctcaggtcgcagacgtttt
gcagcagcagtcgcttcacgttcgctcgcgtatcggtgattcattctgctaaccagtaaggcaaccccgccagcctagc
cgggtcctcaacgacaggagcacgatcatgcgcacccgtggccaggacccaacgctgcccgagatctcgatcccg
cgaaattaatacgactcactatagggagaccacaacggtttccctctagaaataattttgtttaactttaagaaggagata
taccatggatcctctagagtcgacctgcaggcatgcaagcttgcggccacacaggagatagtcatacatgaaacaca
aagaggagaaattaactatgagaggatctcaccatcaccatcaccatacggatccaagcggcctggtgccgcgcgg
cagcatgatcctcgacactgactacataaccgaggatggaaagcctgtcataagaattttcaagaaggaaaacggcg
agtttaagattgagtacgaccggacttttgaaccctacttctacgccctcctgaaggacgattctgccattgaggaagtca
agaagataaccgccgagaggcacgggacggttgtaacggttaagcgggttgaaaaggttcagaagaagttcctagg
gagaccagttgaggtctggaaactctactttactcatccgcaggacgaaccagcgataagggacaagatacgagag
catccagcagttattgacatctacgagtacgacataccttcgccaagcgctacctcatagacaagggattagtgccaa

tggaaggcgacgaggagctgaaaatgctcgccttcgcgattgcgactctctaccatgagggcgaggagttcgccgag
gggccaatccttatgataagctacgccgacgaggaaggggccagggtgataacttggaagaacgtggatctccccta
cgttgacgtcgtctcgacggagagggagatgataaagcgcttcctccgtgttgtgaaggagaaagacccggacgttct
cataacctacaacggcgacaacttcgacttcgcctatctgaaaaagcgctgtgaaaagctcggaataaacttcgccct
cggaagggatggaagcgagccgaagattcagaggatgggcgacaggtttgccgtcgaagtgaagggacggatac
acttcgatctctatcctgtgataagacggacgataaacctgcccacatacacgcttgaggccgtttatgaagccgtcttcg
gtcagccgaaggagaaggtttacgctgaggaaataaccacagcctgggaaaccggcgagaaccttgagagagtcg
cccgctactcgatggaagatgcgaaggtcacatacgagcttgggaaggagttccttccgatggaggcccagctttctcg
cttaatcggccagtccctctgggacgtctcccgctccagcactggcaacctcgttgagtggttcctcctcaggaaggcct
atgagaggaatgagctggccccgaacaagcccgatgaaaaggagctggccagaagacggcagagctatgaagg
aggctatgtaaaagagcccgagagagggttgtgggagaacatagtgtacctagattttagatccctgtacccctcaatc
atcatcacccacaacgtctcgccggatacgctcaacagagaaggatgcaaggaatatgacgttgccccacaggtcg
gccaccgcttctgcaaggacttcccaggatttatcccgagcctgctaggagacctcctagaggagaggcagaagata
aagaagaagatgaaggccacgattgacccgatcgagaggaagctcctcgattacaggcagaggttgatcaagatcc
tggcaaacagctactacggttactacggctatgcaagggcgcgctggtactgcaaggagtgtgcagagagcgtaacg
gcctggggaagggagtacataacgatgaccatcaaggagatagaggaaaagtacggctttaaggtaatctacagcg
acaccgacggatttttgccacaatacctggagccgatgctgaaaccgtcaaaaagaaggctatggagttcctcaagt
atatcaacgccaaacttccgggcgcgcttgagctcgagtacgagggcttctacaaacgcggcttcttcgtcacgaaga
agaagtatgcggtgatagacgaggaaggcaagataacaacgcgcggacttgagattgtgaggcgtgactggagcg
agatagcgaaagagacgcaggcgagggttcttgaagctttgctaaaggacggtgacgtcgagaaggccgtgaggat
agtcaaagaagttaccgaaaagctgagcaagtacgaggttccgccggagaagctggtgatccacgagcagataac
gagggatttaaaggactacaaggcaaccggtccccacgttgccgttgccaagaggttggccgcgagaggagtcaaa
atacgccctggaacggtgataagctacatcgtgctcaagggctctgggaggataggcgacagggcgataccgttcga
cgagttcgacccgacgaagcacaagtacgacgccgagtactacattgagaaccaggttctcccagccgttgagaga
attctgagagccttcggttaccgcaaggaagacctgcgctaccagaagacgagacaggttggtttgagtgcttggctga
agccgaagggaacttgatcgatgctccgagatgaggtaggatggctggcttacggtgttactaatgagccatcctcgag
caccaccaccaccaccactgagatccggctgctaacaaagcccgaaaggaagctgagttggctgctgccaccgctg
agcaataactagcataaccccttggggcctctaaacgggtcttgaggggttttttgctgaaaggaggaactatatccgga
t

[a]All sequences are written in the 5'→3' direction

Table S2. Sequences[a] of the oligonucleotides and templates used in this study.

Category	Name	Sequence[b]
Exo loop InDel mutagenesis	Exo_loop_R	TTCAACTTTGGTGGTGGTTTC
	Exo_loop_INS1	NNSGATTGTCGTGTTTGGGCATATG
	Exo_loop_INS2	NNSNNSGATTGTCGTGTTTGGGCATATG
	Exo_loop_INS3	NNSNNSNNSGATTGTCGTGTTTGGG
	Exo_loop_DEL1	TGTCGTGTTTGGGCATATGG
	Exo_loop_DEL2	CGTGTTTGGGCATATGGCTATATG
	Exo_loop_DEL3	GTTTGGGCATATGGCTATATGAAC
TPR2 loop InDel mutagenesis	TPR2_loop_R	TTCTTTCAGATAAGGAACTTTACC
	TPR2_loop_INS2	NNSNNSAATGGTGCACTGGGT
	TPR2_loop_INS1	NNSAATGGTGCACTGGG
	TPR2_loop_INS3	NNSNNSNNSAATGGTGCACTGGG
	TPR2_loop_DEL1	GGTGCACTGGGTTTTC
	TPR2_loop_DEL2	GCACTGGGTTTTCGTC
Thumb loop InDel mutagenesis	Thumb_loop_R	AACCTGAACCGGTTTCGGTTTC
	Thumb_loop_INS2	NNSNNSCCGGGTGGTGTTGTTC
	Thumb_loop_INS1	NNSCCGGGTGGTGTTGTTCTG
	Thumb_loop_INS3	NNSNNSNNSCCGGGTGGTGTTGTTC

	Thumb_loop_DEL1	GGTGGTGTTGTTCTGGTTGATGATAC
	Thumb_loop_DEL2	GGTGTTGTTCTGGTTGATGATACCTTTAC
	Thumb_loop_DEL3	GTTGTTCTGGTTGATGATACCTTTACGATC
	Thumb_loop_DEL4	GTTCTGGTTGATGATACCTTTACGATCAAA
CST selection	CST_04(7)exoR	/5BiotinTEG/ACC*G*C*A
P562del mutant	p2_thumb_loop_R	AACCTGAACCGGTTTCGGTTTC
	p2_thumb_loop_DEL1	GGTGGTGTTGTTCTGGTTGATGATAC
Exo+THR mutant	iPCR_P2_Exo+_F1	GTATAGCTGCGATTTTGAAACC
	iPCR_P2_Exo+_R1	CTTTTGCGAGGCATGTG
Primer extension assay	TempN-exoR	TGGTCCAGCATCGTGAGATCGATTACCGAA CAGCACTACGTGGCTAAGTGCTTATCTCCTA GCTTAAACGGAT*C*C*G
	TempN_2.7_ExoR	TGGTCCAGCATCGTGAGATCCCTTACTGAA CAGACTACATGGCTAAGTGCTTATCTCCTAG CTTAAACGGAT*C*C*G
RCA assay	P2_RCA_N8_ExoR	NNNNNN*N*N
Fidelity assay	PH_pET23_DA_Biotin 3	/5BiotinTEG/CCCCTTATTAGCGTTTGCCAGC TCTTCCACTCAGGGTtAATGCCAGC
	outnest_1	CCCCTTATTAGCGTTTGCCA
	P2_fidelity_inestR1	CTGTGTGGCCGCAAG
	Fidelity_ref	agggttaatgccagcgcttcgttaatacagatgtaggtgttccac agggtagccagcagcatatggtgcagggcgctgacttccgcgtt

		tccagactttacgaaacacggaaaccgaagaccattcatgttgtt gctcaggtcgcagacgttttgcagcagcagtcgcttcacgttcgc tcgcgtatcggtgattcattctgctaaccagtaaggcaaccccgc cagcctagccgggtcctcaacgacaggagcacgatcatgcgc acccgtggccaggacccaacgctgcccgagatctcgatcccg cgaaattaatacgactcactatagggagaccacaacggtttccc tctagaaataattttgtttaactttaagaaggagatataccatggat cctctagagtcgacctgcaggcatgcaagcttgcggccacaca g

[a]All sequences are written in the 5'→3' direction

[b]N: A/C/G/T; S:G/C

*: phosphorothioate bond

/5BiotinTEG/: Biotin with a 15 atom triethylene glycol (TEG) spacer

Supplementary references

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Rangwala, S. H., Kuznetsov, A., Ananiev, V., Asztalos, A., Borodin, E., Evgeniev, V., Joukov, V., Lotov, V., Pannu, R., Rudnev, D., Shkeda, A., Weitz, E. M., & Schneider, V. A. (2021). Accessing NCBI data using the NCBI sequence viewer and genome data viewer (GDV). *Genome Research*, *31*(1), 159–169. https://doi.org/10.1101/gr.266932.120

Torres, L. L., & Pinheiro, V. B. (2018). Xenobiotic Nucleic Acid (XNA) Synthesis by Phi29 DNA Polymerase. *Current Protocols in Chemical Biology*, *10*(2), e41. https://doi.org/10.1002/cpch.41